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SAGE-Hindawi Access to Research
International Journal of Alzheimer's Disease
Volume 2011, Article ID 505984, 11 pages
doi:10.4061/2011/505984

Research Article

Alzheimer's Disease Genes Are Associated with Measures of Cognitive Ageing in the Lothian Birth Cohorts of 1921 and 1936

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Received 24 November 2010; Revised 23 February 2011; Accepted 27 March 2011

Academic Editor: Lars Bertram

Alzheimer's disease patients have deficits in specific cognitive domains, and susceptibility genes for this disease may influence human cognition in nondemented individuals. To evaluate the role of Alzheimer's disease-linked genetic variation on cognition and normal cognitive ageing, we investigated two Scottish cohorts for which assessments in major cognitive domains are available: the Lothian Birth Cohort of 1921 and the Lothian Birth Cohort of 1936, consisting of 505 and 998 individuals, respectively. 158 SNPs from eleven genes were evaluated. Single SNP analyses did not reveal any statistical association after correction for multiple testing. One haplotype from *TRAPPC6A* was associated with nonverbal reasoning in both cohorts and combined data sets. This haplotype explains a small proportion of the phenotypic variability (1.8%). These findings warrant further investigation as biological modifiers of cognitive ageing.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease, and it is predicted to affect over a million people in the UK by 2025 (Dementia UK 2007 report). AD is characterised initially by impaired episodic memory [1] and, as the disease progresses, other cognitive deficits appear, particularly in attention and executive functions, semantic memory, language, and spatial orientation [2, 3].

AD is a genetically heterogeneous disease. Mutations in three genes (the amyloid precursor protein, *APP*; presenilin 1, *PS1*; presenilin 2, *PS2*) are known to cause a rare early-onset form of AD [4–6]. The most common form of AD occurs sporadically and with a late age at onset. Until recently, the only well-replicated risk factor for this form of AD was the $\varepsilon 4$ allele of the apolipoprotein E (*APOE*) gene [7]. However, three recent genome-wide association studies (GWASs) have identified four new candidate genes for sporadic AD—*BIN1, CLU, CR1,* and *PICALM*—and one new genomic region near *BLOC1S3/EXOC3L2/MARK4* [8–10]. Associations with *CLU, CR1,* and *PICALM* have been replicated [11–13].

Nonpathological age-related cognitive decline is a major and growing concern in developed societies [14]. General cognitive ability is an important predictor of life outcomes, including in old age. The determinants of normal cognitive ageing are not fully understood, but are likely to include both genetic and environmental influences [14]. Genetic influences on cognitive ability increase from about 30% in childhood to as much as 80% in later adulthood, and these decrease slightly in very old-age when, probably, stochastic effects become relatively more important [15]. As is still

Table 1: Details of the Lothian Birth Cohorts of 1921 and 1936.

	LBC1921	LBC1936
Total	505	998
Females (%)	296 (58.7)	494 (49.5)
Males (%)	209 (41.3)	504 (50.5)
Mean age in years[1] (± s.d)	10.9 ± 0.28	10.9 ± 0.28
Mean age in years[2] (± s.d)	79.11 ± 0.57	69.58 ± 0.83
≥1 *APOE ε*4 allele	135	287
no *APOE ε*4 allele	370	672

[1]Mean age at original test date, [2]Mean age when revisited.

true for many complex phenotypes, there are few replicated genotype-phenotype associations with cognitive ageing [15, 16]. There is suggestive evidence for genes such as *BDNF* and *COMT* but, to date, *APOE* is the only gene that has been consistently shown to have a significant, but small, influence on age-related cognitive decline [17]. We hypothesise that other genes involved in AD may play a role in normal cognitive ageing. Indeed, a recent study has described the association of variants in the *CLU* and *PICALM* genes with cognitive function [18].

Here, we examine genetic variants from the *APP*, *PS1*, *PS2, BIN1, CLU, CR1, PICALM* genes, and the region surrounding the *BLOC1S3/EXOC3L2/MARK4* genes on chromosome 19 in two large, phenotypically well-defined cohorts, the Lothian Birth Cohorts of 1921 and 1936 [19, 20]. The individuals in these cohorts took a general mental ability test in childhood and then took a range of mental tests in old age. They are, therefore, unusually useful in understanding the genetic contributions to cognitive change across most of the human life course. The *APOE* gene has previously been investigated in these cohorts and shown to explain a small percentage (0.005–0.01) of the variance associated with the general cognitive factor, two nonverbal tests, and choice reaction time variability [21–24].

2. Materials and Methods

2.1. Sample. The samples examined were the Lothian Birth Cohort of 1921 (LBC1921) and the Lothian Birth Cohort of 1936 (LBC1936). They were born in 1921 and 1936, respectively and, at a mean age of 11 years, they were tested on general cognitive ability by means of the Scottish Mental Survey of 1932 (SMS1932) or the Scottish Mental Survey of 1947 (SMS1947) (each cohort has a mean age = 10.9 ± 0.28 years). Since 1999 for LBC1921 and 2004 for LBC1936, a number of the original Surveys' participants who were living in the Edinburgh area of Scotland have been revisited. Participants from LBC1921 were tested for a variety of cognitive phenotypes at approximately 79 years of age (mean age = 79.11 ± 0.57 years), whereas participants from the LBC1936 were tested at approximately 70 years of age (mean age = 69.58 ± 0.83 years) (Table 1) [19, 20].

Individuals were excluded from this study if there was a personal history of dementia, if they had an MMSE score of less than 24, or if they did not have GWAS data. Four individuals were removed from the LBC1921 due to a family history of dementia, and eight were removed due to MMSE < 24. Seven individuals were removed from the LBC1936 with MMSE < 24. The total number of participants included from the LBC1921 was 505 (41.3% male: 58.7% female), and the total number of participants from the LBC1936 was 998 (50.5% male: 49.5% female) (Table 1).

The LBC1936 was used as the discovery cohort. Significant results meeting the chosen statistical criteria were carried forward and investigated using the LBC1921.

2.2. Cognitive Tests. Individuals from the LBC1936 were tested on the Moray House Test (MHT) no. 12 at age 11 (10.9 ± 0.28 years) and subsequently at age 70 (69.58 ± 0.83 years) [19]. At age 70, they were also tested for a variety of cognitive phenotypes, with the ones of interest to this study being verbal fluency (a test of executive function using the letters C, F, and L) [25], matrix reasoning (a subtest from the Wechsler Adult Intelligence Scale-III^{UK} used to assess nonverbal reasoning) [26], and logical memory (a test of immediate and delayed verbal declarative memory from the Wechsler Memory Scale-III^{UK}) [27].

Individuals from the LBC1921 were tested on the MHT no. 12 at age 11 (10.9 ± 0.28 years) and subsequently at age 79 (79.11 ± 0.57 years) [20]. This cohort's participants were tested for three cognitive phenotypes; verbal fluency (exactly as applied in the LBC1936), Raven's Standard Progressive Matrices (a test of non-verbal reasoning) [28], and logical memory (a test of immediate and delayed verbal declarative memory from the Wechsler Memory Scale-Revised [29].

From this point forward, age 11 for both cohorts indicates 10.9 ± 0.28; age 70 for the LBC1936 cohort indicates 69.58 ± 0.83 years; age 79 for the LBC1921 cohort indicates 79.11 ± 0.57 years.

2.3. Genotyping. Genomic DNA from the LBC1936 cohort was isolated from whole blood by standard procedures at the Wellcome Trust Clinical Research Facility (WTCRF), Genetics Core, Western General Hospital, Edinburgh. Genomic DNA from the LBC1921 cohort was isolated from whole blood by standard procedures at Medical Research Council (MRC) Technology, Western General Hospital, Edinburgh. All samples were genotyped at the WTCRF Genetics Core with the Illumina Human 610-Quadv1 chip as part of a larger study [30]. SNPs were included in the analyses if they met the following conditions: call rate ≥ 0.98, minor allele frequency ≥ 0.01, and Hardy-Weinberg Equilibrium test with $P \geq .001$ [30]. For this study, specific SNPs were selected from the GWAS data set. Genomic regions approximately 5 kb upstream to 5 kb downstream of each candidate gene were identified using positional information from the Santa Cruz Genome Browser, March 2006 Assembly (NCBI36) (http://genome.ucsc.edu/) [31]. All SNPs with available genotype data from each region were used in this study. A further five SNPs that showed association with sporadic AD were included: four that were outside the above genomic regions and one that was within the genomic region but that had not been genotyped. This SNP (rs6656401)

was imputed using the HapMap phase II CEU data (NCBI build 36 (UCSC hg18)) as the reference sample and MACH software. The imputation quality score for this SNP was high ($r^2 = 0.92$). A total of 158 SNPs were selected; *66* from APP, 9 from *PS1*, 6 from *PS2,* 17 from *BIN1*, 6 from *CLU,* 9 from *CR1,* 29 from *PICALM*, and 16 from the *BLOC1S3/EXOC3L2/MARK4* region, which included three SNPs from the 5′ end of *TRAPPC6A* gene (Table S1). *APOE* haplotype data were available for all samples.

2.4. Statistical Analysis

2.4.1. Significance Threshold. To determine the correct level of significance for regression and haplotype analyses of the LBC1936 cohort, a spectral decomposition program, SNPSpD, was used [9]. SNPSpD calculates an approximate estimate of the effective number of independent SNPs using a previously described method [32]. A Bonferroni calculation using this number of SNPs was used to determine the appropriate level of significance for regression and haplotype analysis. A significance level for pairwise interaction analyses of the LBC1936 cohort was determined using $\alpha = 0.05/x$, where $x = n(n-1)/2$ (n = effective number of independent SNPs) [33].

2.4.2. Cognitive Phenotypes. Standardized residual scores were calculated for each cognitive phenotype to incorporate age at time of testing and gender, using linear regression in SPSS, v14.0.

2.4.3. Association Analysis. Unless otherwise noted, all statistical analyses were carried out using PLINK v1.07 (http://pngu.mgh.harvard.edu/purcell/plink) [34]. Three approaches to association analysis were used.

The first approach examined all SNPs in relation to the selected cognitive phenotypes and applied a stringent Bonferonni threshold to the P values. Linear regression analysis was performed under an additive model in PLINK. Additional analyses included two covariates; (i) the presence or absence of an *APOE ε4* allele and (ii) general cognitive ability at age 11 (MHT score adjusted for age) to adjust for prior cognitive ability. Using general cognitive ability at age 11 as a covariate enables the role of each SNP in cognitive ageing to be explored. Two stratified data sets, with or without the *APOE ε4* allele, were analysed similarly. Adaptive permutation analysis was carried out on all linear regression analyses.

The second approach was haplotype analysis. Each gene was examined for association with cognitive phenotypes using a sliding window of three SNPs, shifting one SNP at a time. Two stratified data sets, with or without the *APOE ε4* allele, were analysed similarly. In the haplotype analysis, the presence or absence of an *APOE ε4* allele and general cognitive ability at age 11 (MHT score adjusted for age) were not used as covariates. SNP regions meeting the significance threshold were analysed using max(T), a label swapping-based permutation method.

The third and final approach used pairwise interaction analysis to determine any effect of gene-gene interaction on the association with cognitive phenotypes. The full data set and two stratified data sets, with or without the *APOE ε4* allele, were analysed similarly. In the pairwise interaction analysis, the presence or absence of an *APOE ε4* allele and general cognitive ability at age 11 (MHT score adjusted for age) were not used as covariates. The results file was controlled so that only associations having $P \leq .0001$ were reported. Additionally, only where SNPs were located in different genes are the pairwise interactions described here. Significant interactions were analysed using a one-way ANOVA in SPSS v14.0. To examine each interaction, both the cognitive mean of each genotype (aabb, aaBB, aaBb, AAbb, Aabb, AABB, AABb, AaBB, AaBb) and the cognitive mean of the groups representing the presence or absence of each minor allele (aabb, aaB-, A-bb, A-B-) were compared (where a and b represent the minor allele of each SNP).

2.4.4. Linkage Disequilibrium Analysis. Linkage disequilibrium (LD) values were generated and visualised using Haploview [35].

3. Results

3.1. Significance Threshold. 158 SNPs in total were selected for analysis in this study (Table S1 in Supplementary Material available online at doi:10.4061/2011/505984). The LBC1936 cohort was used as a discovery sample and the LBC1921 cohort as a replication cohort. Different significance thresholds were applied to each cohort. To determine an appropriate threshold for analyses of the discovery cohort, two methods were used. Spectral decomposition analysis calculated that the approximate estimate of the effective number of independent SNPs was 89.24. Therefore, in our regression and haplotype analyses, only where $P \leq .00056$ ($\alpha = 0.05/89.24$), were results considered significant associations. For pairwise interaction analysis, only where $P \leq .000013$ ($\alpha = 0.05/x$, x = [89.24 (89.24-1)]/2) were results considered significant associations. max(T) permutation analysis was carried out on significant haplotype results, and a significance threshold of $P \leq .05$ was applied to the results. Results with $P \leq .05$ were considered significant in our replication cohort.

3.2. Association of AD SNPs with Cognitive Phenotypes. No individual SNP in the LBC1936 was associated with any cognitive phenotype in the overall or *APOE* stratified sample at $P \leq .00056$ (Table S2, Table S3).

3.3. Association of AD Gene Blocks with Cognitive Phenotypes. Tables S4, S5, and S6 detail the effect of each 3-SNP window on each cognitive phenotype in the complete LBC1936 data set and in the LBC1936 data sets stratified for presence or absence of the *APOE ε*4 allele.

Two 3-SNP windows, comprising four adjacent SNPs from *BIN1*, reached our corrected P value level ($P \leq .00056$) with general cognitive ability at age 11 (MHT adjusted) in

the overall LBC1936 sample (Table 2). These results were not replicated in the LBC1921 and were nonsignificant following permutation analysis of both the LBC1936 and the combined data set.

Two separate 3-SNP windows from the *APP* locus reached significance with logical memory in the *APOE* $\varepsilon 4$ positive subgroup (Table 2). These SNP windows were not significant postpermutation analysis of the LBC1936. Further, this result was not replicated in the LBC1921 or following permutation analysis of the combined sample.

One 3-SNP window from the *TRAPPC6A* locus reached significance with matrix reasoning in the *APOE* $\varepsilon 4$ negative subgroup (Table 2). Though not significant postpermutation analysis in the LBC1936, this finding was replicated in the LBC1921 and in post permutation analysis of the combined cohort.

3.4. Gene-Gene Interaction Analysis. Tables S7 11 detail the results obtained in the pairwise interaction analyses with each cognitive phenotype in the LBC1936. Data were extracted for interactions if $P \leq .0001$. Results were considered significant if $P \leq .000013$.

One SNP-SNP interaction from the chromosome 19 locus (*MARK*4, rs344807) and *APP* (rs12482753) was significantly associated with general cognitive ability at age 70 (MHT adjusted for age) in the *APOE* $\varepsilon 4$ negative LBC1936 subset (Figure 1; Table 3). However, analysis of the cognitive means for each genotype group indicated that the association was due to the low score of a single individual who expressed the aaBb genotype. Analysis of the cognitive means of the four groups representing the presence or absence of the minor alleles showed no significant difference, and following the removal of the aaBb individual the genotype result was no longer significant (results not shown). This interaction was not replicated in the LBC1921.

A single SNP-SNP interaction from *PS1* (rs214260) and *APP* (rs440666) was significantly associated with verbal fluency in the *APOE* $\varepsilon 4$ negative LBC1936 subset (Figure 1; Table 3). Analysis of the cognitive means for each genotype group indicated that the association was due to the lower verbal fluency scores of the group expressing the Aabb genotype; however, analysis of the cognitive means of the four groups representing the presence or absence of the minor alleles showed no significant difference (results not shown). This interaction was not replicated in the LBC1921.

One SNP-SNP interaction from *BIN1* (rs10200967) and *APP* (rs2830036) was significantly associated with verbal declarative memory in the *APOE* $\varepsilon 4$ positive LBC1936 subset (Figure 1; Table 3). Analysis of the cognitive means for each genotype and for the four groups representing the presence or absence of the minor alleles indicated that this association was due to the low logical memory scores of two individuals homozygous for each minor allele (Figure 2). Although not a direct replication of the result observed in the LBC1936 cohort, two *BIN1-APP* interactions approached significance in the LBC1921 cohort. The associations were observed with the *BIN1* SNP (rs10200967) that was associated in the LBC1936 *APOE* $\varepsilon 4$ positive sample set, but with two different *APP* SNPs: rs396969 and rs383700 (Table 3). The two *APP* SNPS were in complete LD (Figure 1). Both interactions were associated with higher logical memory scores, with the opposite of that observed in the LBC1936. Analysis of the cognitive means for each genotype indicated that both associations were due to the high logical memory score of one individual homozygous for each minor allele. Following the removal of this individual, this result was no longer significant (results not shown). No *BIN1-APP* SNP interactions were observed in the *APOE* $\varepsilon 4$ positive samples in LBC1921, and there was no significant interaction when the samples were combined.

A single SNP-SNP interaction from *PS2* (rs1150895) and *PICALM* (rs3851179) was significantly associated with verbal declarative memory in the *APOE* $\varepsilon 4$ negative LBC1936 subset (Figure 1; Table 3). Analysis of the cognitive means for each genotype group indicated that the association was due to the higher logical memory scores of the groups expressing either the AAbb or aaBB genotype compared to the AABB genotype. Further analysis of the cognitive means of the four groups representing the presence or absence of the minor alleles showed that aaBB and aaBb individuals had higher logical memory scores than other allele groups (results not shown). This interaction was not replicated in the LBC1921.

4. Discussion

In this study, we have screened polymorphisms from three causal and five putative risk genes for Alzheimer's disease in two cohorts with extensive and unique cognitive phenotypes available. Evidence was found to suggest a role for variation in a gene at the chromosome 19 locus, *APP* and *BIN1* in cognitive ability.

Each gene will be discussed individually.

4.1. Chromosome 19 Locus. A genomic locus on chromosome 19 was recently implicated in a single LOAD-GWAS [10]. It identified a locus distal to and not in linkage disequilibrium with *APOE*. The SNPs chosen in this study span the 5′ end of the *TRAPPC6A* gene and cover *BLOC1S3*, *EXOC3L2, MARK4,* and the 3′ end of the *CKM* gene.

One 3-SNP window located at the 5′ end of the *BLOC1S3/EXOC3L2/MARK4* region was significantly associated with non-verbal reasoning in individuals lacking an *APOE* $\varepsilon 4$ gene in the LBC1936 data set. This SNP window consisted of the SNPs (rs7247764, rs28555639, rs12460041) located at the 5′ end of the *TRAPPC6A* gene. They span a genomic region of 1442 bp and are in complete LD ($D' = 1$). The genotype of this associated haplotype was TTT, and it was the most common haplotype ($f = 0.70$). This haplotype was associated with a small decrease in Wechsler matrix reasoning scores ($\beta = -0.21$) and explained 1.8% of the variation in the LBC1936. This was replicated in the LBC1921 cohort ($\beta = -0.18$), where it explained 1.3% of the variation in Raven's Standard Progressive Matrices scores. Permutation analysis of the combined data set confirmed this result.

Table 2: Significant haplotype results.

Gene	dbSNP ID (rs)	Haplotype	Cohort	Sample	*N*	Frequency	Cognitive phenotype	Beta	r^2	*P*	max(T)
BIN1	3768857/17014873/2276575	GAG	LBC1936	Overall	941	0.13	GCA11	−0.24	0.012	.00048*	0.21
		GAG	LBC1921	Overall	453	0.13	GCA11	−0.11	0.0031	.24	
		GAG	Both	Overall	1394	0.13	GCA11	−0.19	0.0091	.00036	0.14
	17014873/2276575/13430599	AGT	LBC1936	Overall	941	0.13	GCA11	−0.24	0.014	.0003*	0.16
		AGT	LBC1921	Overall	453	0.13	GCA11	−0.12	0.003	.23	
		AGT	Both	Overall	1394	0.13	GCA11	−0.2	0.0096	.00025	0.1
APP	2829997/440666/2014146	GTG	LBC1936	*APOE* ε4 positive	287	0.013	LM	−1.3	0.043	.0004*	0.18
		GTG	LBC1921	*APOE* ε4 positive	134	0.011	LM	−0.051	0.00004	.94	
		GTG	Both	*APOE* ε4 positive	421	0.012	LM	−1	0.023	.0017	0.49
APP	1783025/380417/1787438	TTG	LBC1936	*APOE* ε4 positive	287	0.053	LM	0.72	0.048	.00017*	0.072
		TTG	LBC1921	*APOE* ε4 positive	134	0.053	LM	0.042	0.00016	.88	
		TTG	Both	*APOE* ε4 positive	421	0.053	LM	0.516	0.024	.0014	0.43
TRAPPC6A	7247764/28555639/12460041	TTT	LBC1936	*APOE* ε4 negative	669	0.7	MR	−0.21	0.018	.00043*	0.24
		TTT	LBC1921	*APOE* ε4 negative	369	0.71	MR	−0.18	0.013	.024**	
		TTT	Both	*APOE* ε4 negative	1039	0.7	MR	−0.2	0.016	.000036	0.019***

A result is significant with the LBC1936 cohort if $P \leq .00056$ (*) and with the LBC1921 cohort if $P \leq .05$ (**). A result is significant postpermutation analysis if $P \leq .05$ (***). The following abbreviations are used: *N*, sample number; Beta, regression coefficient of the trait value; r^2, proportion of the variance explained; GCA11, general cognitive ability at age 11 (MHT adjusted for age); LM, logical memory; MR, matrix reasoning. max(T) *P* value is controlled for all SNPs tested.

Table 3: Significant pairwise interaction results.

Cohort	Samples	Gene 1	dbSNP ID (rs)	Gene 2	dbSNP ID (rs)	Cognitive phenotype	Beta	*P*
LBC1936	*APOE* ε4 negative	*MARK4*	344807	*APP*	12482753	GCA70	−1.69	.000012*
LBC1921	*APOE* ε4 positive	intergenic chr 19	2627641	*APP*	2829984	GCA70	−1.21	.000032
		intergenic chr 19	597668	*APP*	2829984	GCA70	−1.21	.000032
Both	*APOE* ε4 negative	*MARK4*	344807	*APP*	12482753	GCA70	−1.27	.000056
LBC1936	*APOE* ε4 negative	*PS1*	214260	*APP*	440666	VF	−0.5	.000012*
LBC1936	*APOE* ε4 negative	*PS2*	1150895	*PICALM*	3851179	LM	−0.43	.0000048*
LBC1936	*APOE* ε4 positive	*BIN1*	10200967	*APP*	2830036	LM	−0.67	.000011*
LBC1921	Overall	*BIN1*	10200967	*APP*	396969	LM	0.62	.00033
		BIN1	10200967	*APP*	383700	LM	0.62	.00032

A result is significant with the LBC1936 cohort if $P \leq .000013$ (*) and with the LBC1921 cohort if $P \leq .05$ (**). The following abbreviations are used: *N*, sample number; Beta, regression coefficient of the trait value; GCA70, general cognitive ability at age 70 (MHT adjusted for age); VF, verbal fluency; LM, logical memory.

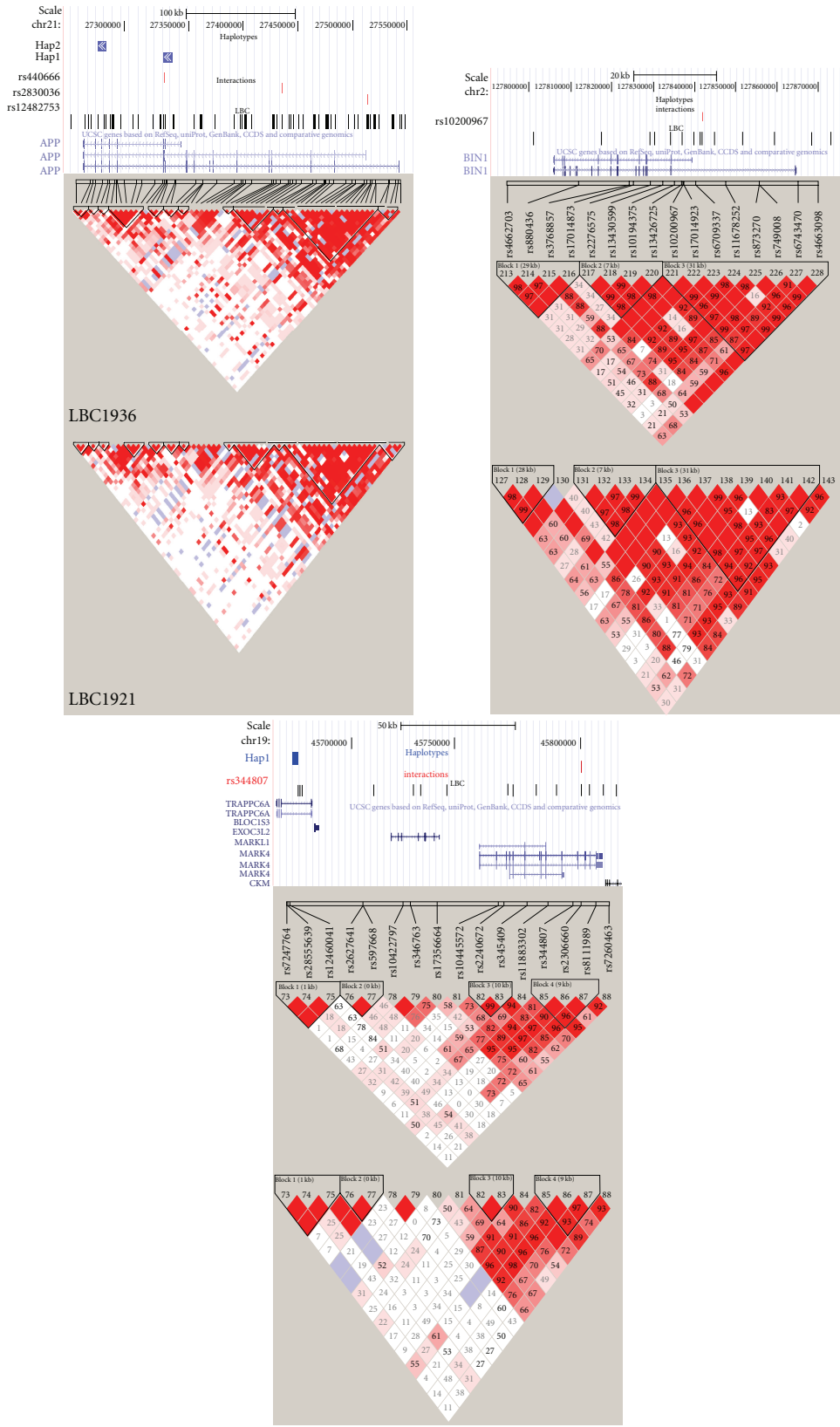
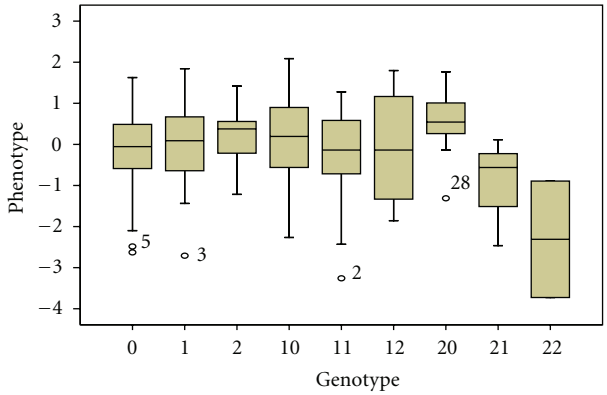


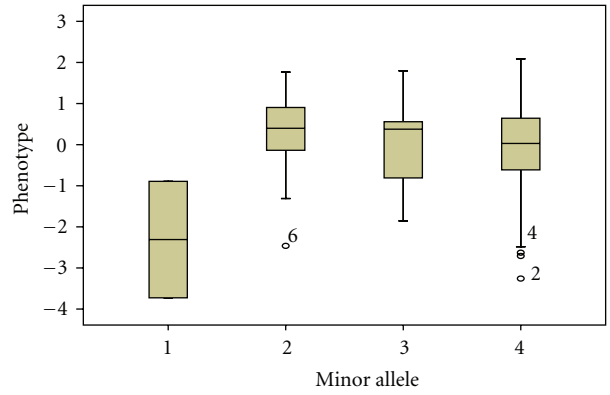
Figure 1: Genomic structure of positively associated genes. (a) Genomic structure of *APP*, *BIN1*, and chromosome 19. Highlighted are the location of each SNP genotyped and the location of positively associated haplotypes and gene-gene interactions. (b) LD structure of *APP*, *BIN1*, and chromosome 19 in the Lothian Birth Cohorts of 1936 (top) and 1921 (bottom). LD values used were D'.

	Sum of squares	*df*	Mean square	*F*	Sig.
Between groups	21.611	8	2.701	2.96	.003
Within groups	252.824	277	.913		
Total	274.434	285			



(a)

	Sum of squares	*df*	Mean square	*F*	Sig.
Between groups	11.58	3	3.86	4.141	.007
Within groups	262.854	282	.932		
Total	274.434	285			



(b)

Figure 2: The interaction of an SNP pair from *BIN1* and *APP* is likely to influence logical memory in the *APOE* ε4 positive subset of LBC1936. Analysis of both the (a) genotype cognitive means and (b) the allele specific means shows that the initial positive result is due to two individuals carrying both minor alleles, aabb. Genotype legend; 11 = AaBb, 10 = AaBB, 01 = AABb, 00 = AABB, 12 = Aabb, 01 = AAbb, 21 = aaBb, 20 = aaBB, 22 = aabb. Allele legend; 1 = aabb, 2 = aaB-, 3 = A-bb, 4 = A-B-.

The SNP associated with LOAD in the recent GWAS study [10], rs597668, is located in an intergenic region between *TRAPPC6A* and *EXOC3L2*. This SNP was included in our study although we did not observe an association with any cognitive phenotype. The *TRAPPC6A* haplotype is located 31573 bp from the GWAS SNP, and analysis of the LD in this region shows that SNPs from the haplotype were not in the same LD block as the GWAS SNP ($D' = 0.22$), so it is unclear whether our results are detecting the same effect. Replication of the *TRAPPC6A* haplotype is required in a larger cohort.

4.2. APP. *APP* was the first disease gene identified in familial AD [4]. It is a transmembrane protein, and sequential cleavage by β- and γ-secretase releases the β-amyloid peptide. Although the exact role of the *APP* protein is unknown, it is considered central to AD pathogenesis.

Two 3-SNP windows at the *APP* locus, each consisting of three SNPs, were associated with verbal declarative memory in individuals carrying at least one *APOE* ε4 allele in the LBC1936. These results correspond to two genomic regions located at the 3' end of the *APP* gene. The first region consisted of three SNPs, rs2829997, rs440666, and rs2014146, and spanned 8163 bp. These SNPs were in high LD ($D' > 0.7$) and constituted a haplotype block. The associated haplotype, with genotype GTG, was rare, with a frequency of 0.013. This haplotype, *APP* Hap1, was associated with a decrease in logical memory scores ($\beta = -1.312$) and explained 4.3% of the variation. The second genomic region spanned 7326 bp and consisted of three SNPs, rs1783025, rs380417, and rs1787438. These SNPs are located near known pathogenic AD mutations, in sites encoding the α, β, and γ-secretase sites. The latter two SNPs were in complete LD ($D' = 1$); however, rs1783025 was not (D' 0.48, 0.64 with rs380417, rs1787438, respectively). The associated genotype, TTG, was rare, with a frequency of 0.053. This genotype was associated with an increase in logical memory scores ($\beta = 0.72$) and explained 4.8% of the variation. These two genotypes explain a small, but important, amount of the variance, 4.3% and 4.8% respectively, especially considering that *APOE* ε4 contributes 0.5–1% to variance in cognitive traits. However, these results were not replicated following permutation analysis. Further, this effect was not observed in the LBC1921 or in the combined data set.

These results may not have been replicated in the LBC1921 cohort for a couple of reasons: the replication cohort contains fewer individuals and the logical memory test used with the LBC1921 cohort differed slightly from that used with the LBC1936 cohort. Nonetheless, the haplotype frequencies are consistent between cohorts and, although not significant, LBC1921 individuals with *APP* Hap1 (GTG) have lower logical memory scores while individuals with the second associated genotype (TTG) have higher logical memory scores in the LBC1921.

Further evidence of a role for *APP* in logical memory was obtained in our gene-gene interaction analysis. SNPs at the *APP* locus were observed to statistically interact with polymorphisms at the *BIN1* locus to influence verbal declarative memory.

4.3. BIN1. *BIN1* was identified as a putative risk factor for LOAD in a recent GWAS study [10]. It encodes several

Table 4: Comparison of significant findings between studies.

Gene	Paper	Genotyping method	SNP (rs)	*P* value	OR	β	Trait
CLU	Harold et al. [8]	Illumina 610 quad Illumina Human Hap550/300	11136000**	8.5×10^{-10}	0.86		LOAD
	Lambert et al. [9]	Illumina 610 quad	11136000	7.5×10^{-9}	0.86		LOAD
	Carrasquillo et al. [11]	Taqman	11136000	8.6×10^{-5}	0.82		LOAD
	Corneveaux et al. [12]	Genome-wide Human SNP6.0 array, Affymetrix	11136000	0.04	0.86		LOAD
	Kamboh et al. [13]	Taqman	11136000	4.4×10^{-16}	0.86		LOAD
	Mengel-From et al. [18]	Taqman	11136000	0.016		0.5	CCS
PICALM	Harold et al. [8]	Illumina 610 quad Illumina Human Hap550/300	3851179**	1.3×10^{-9}	0.86		LOAD
	Carrasquillo et al. [11]	Taqman	3851179	1.3×10^{-5}	0.8		LOAD
	Kamboh et al. [13]	Taqman	3851179	3.4×10^{-9}	0.88		LOAD
	Mengel-From et al. [18]	Taqman	3851179	0.024		1.4	CCS*
	Hamilton et al. 2011	Illumina 610 quad v1.0	3851179 (interaction with *PS2*)	0.0000048		−0.43	LM
	Corneveaux et al. [12]	Genome-wide Human SNP6.0 array, Affymetrix	541458**	0.01	0.81		LOAD
	Kamboh et al. [13]	Taqman	541458	3.5×10^{-9}	0.87		LOAD
CR1	Lambert et al. [9]	Illumina 610 quad	6656401**	3.7×10^{-9}	1.21		LOAD
	Corneveaux et al. [12]	Genome-wide Human SNP6.0 array, Affymetrix	6656401	0.008	1.28		LOAD
	Kamboh et al. [13]	Taqman	6656401	2.3×10^{-9}	1.17		LOAD
	Carrasquillo et al. [11]	Taqman	3818361**	0.014	1.15		LOAD
	Kamboh et al. [13]	Taqman	3818361	5.2×10^{-13}	1.21		LOAD
BIN1	Seshadri et al. [10]	Illumina 610 quad 6.0 (amongst others)	744373**	1.6×10^{-11}	1.13		LOAD
BIN1	Hamilton et al. 2011	Illumina 610 quad v1.0	10200967 (interaction with *APP*)	0.000011		−0.67	LM
chr19	Seshadri et al. [10]	Illumina 610 quad 6.0 (amongst others)	597668**	6.4×10^{-9}	1.18		LOAD
	Hamilton et al. 2011	Illumina 610 quad v1.0	7247764, 28555639, 12460041	0.000036		0.016	MR
	Hamilton et al. 2011	Illumina 610 quad v1.0	344807 (interaction with *APP*)	0.000012			GCA70

Results are provided from recent GWAS for sporadic AD and compared to the results obtained in this study. The following abbreviations are used: OR, odds ratio; Beta, regression coefficient of the trait value; GCA70, general cognitive ability at age 70 (MHT adjusted for age); LM, logical memory; MR, matrix reasoning; LOAD, late-onset Alzheimer's disease; CCS, cognitive composite score; n/a, not applicable. *observed in males. **included in the LBC1921 and LBC1936 study.

isoforms that are expressed in the central nervous system and may be involved in synaptic vesicle endocytosis.

An interaction between rs10200967 (*BIN1*) and rs2830036 (*APP*) was significantly associated with verbal declarative memory in the *APOE* $\varepsilon 4$ positive LBC1936 subset. Further analysis showed that this was due to the low logical memory scores of two individuals expressing both minor alleles of rs10200967 (C, *BIN1)* and rs2830036 (T, *APP*) (Figure 2). This result was not replicated in the LBC1921 cohort or in the combined analysis.

However, these results are consistent with the association of *APP* Hap1, GTG, which is associated with a similar decrease in logical memory scores in the *APOE* $\varepsilon 4$ positive subset of LBC1936. The *APP* SNP involved in the *APP-BIN1* interaction (rs2830036) is located 5′ to *APP* haplotype 1 but there are low levels of LD between them ($D' = 0.34$). Indeed the two individuals contributing to the interaction association do not carry the *APP* Hap1 genotype associated with a decrease in logical memory scores (*APP* Hap1 genotype, GTG; individual genotypes, both AA-CC-AG).

Although the *BIN1-APP* interaction was not replicated in the LBC1921, an association approaching significance was observed with variants from *APP* and *BIN1* and verbal declarative memory in the overall LBC1921 cohort. This was due to the higher logical memory score of a single individual expressing both minor alleles of the two SNPs (*BIN1,* rs10200967; *APP,* rs396969 and rs383700), so may not hold up in a replication study. The two *APP* SNPs involved in this interaction were in LD ($D' = 0.98$) with the second *APP* region, genotype TTG, which was associated with higher logical memory scores in the *APOE* $\varepsilon 4$ positive subset of LBC1936. Again, the individual responsible for the interaction result did not carry the haplotype associated with increased logical memory scores (*APP* region 2 genotype, TTG; individual genotype, CT-TT-TT).

The two *BIN1* SNPs involved in the association of *APP-BIN1* with verbal declarative memory (rs10200967 and rs4663098) are located near to the 5′ end of the *BIN1* gene. There is high LD in this region of *BIN1*, and the SNP associated with LOAD in the recent GWAS, rs744373, is located 21580 bp 5′ of rs4663098 ($D' = 0.93$). There are no current reports of an *in vivo* interaction between *BIN1* and *APP.* However, *APP* is a transmembrane protein and is transported through the secretory pathway. It is possible that through its role in endocytosis, *BIN1* may interact with *APP.*

5. Conclusions

This study indicates that gene specific variation and gene-gene interactions may influence cognition. Our strongest results implicate a role for a haplotype at the *TRAPPC6A* locus in non-verbal reasoning in individuals lacking the *APOE* $\varepsilon 4$ allele. A less clear role for *APP* and *BIN1* in influencing verbal declarative memory in individuals carrying at least one *APOE* $\varepsilon 4$ allele is suggested.

The effect sizes we have observed in this study are small. Indeed, despite the comparability of genomic LD structure, the majority of these associations were not replicated in the LBC1921 cohort. However, it should be noted that the replication cohort ($n = 505$) is smaller than the discovery cohort ($n = 998$). Particularly, our main results were observed in the smaller *APOE* stratified groups. In addition, the individuals in each cohort were retested at different ages; the LBC1921 were re-tested at age 79, while the LBC1936 were re-tested at age 70, and not all cognitive tests used were all identical, although they were similar.

The results presented here were obtained with SNPs not previously associated with sporadic AD, suggesting that either allelic heterogeneity or a functional SNP is not yet identified (Table 4). Nonetheless, the results presented here identify interactions between recently identified and previously known AD genes and provide an interesting insight into potential molecular pathways underlying cognitive traits. They require further investigation in larger identically phenotyped cohorts.

Acknowledgments

G. Hamilton is an Alzheimer's Society Fellow. The authors thank the cohort participants who contributed to these studies. Genotyping of the cohorts was supported by the UK's Biotechnology and Biological Sciences Research Council (BBSRC). Phenotype collection in the Lothian Birth Cohort 1921 was supported by the BBSRC, The Royal Society, and The Chief Scientist Office of the Scottish Government. Phenotype collection in the Lothian Birth Cohort 1936 was supported by Research Into Ageing (continues as part of Age UK's The Disconnected Mind project). The work was supported by The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (G0700704/84698). Funding from the BBSRC, EPSRC, ESRC, and MRC is gratefully acknowledged.

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